Changes in Rat Liver Plasma Membrane Phospholipids During Aging (37163)

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(Introduced by M. Lipkin)
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Decreasing levels of phosphatidyl ethanolamine were noted by Kritchevsky and Howard (1) in human diploid cells maintained through several passages in cell culture. These investigators suggested that cellular phosphatidyl ethanolamine and phosphatidyl inositol levels decreased with age, while phosphatidyl choline increased. Parenchymal liver cells of adult nongrowing mice (and probably rats as well) belong to a slowly renewing population with an average lifetime approaching the lifespan of the animal (2). The phospholipid distributions of total liver and isolated liver plasma membranes were determined on livers obtained from rats of various sizes to compare the changes associated with aging *in vivo* with those observed (1) in tissue culture.

Male CD rats of various sizes and ages were obtained from the Charles River Breeding Laboratory and maintained on Purina chow and water *ad libitum*. Rats were fasted for 16 hr before sacrifice. The pooled livers of at least 2 rats were used for lipid extraction or membrane preparation. Liver plasma membranes were prepared by the procedure of Neville (3), and were characterized on the basis of electron microscopy of fixed preparations and enzyme assay. Little or no rough endoplasmic reticulum and essentially no mitochondria were visible in electron microscopy. Total ATPase and 5′-nucleotidase activity in membranes was approximately equivalent in rats of all age groups. Cytochrome oxidase and glucose-6-phosphatase activities were such that less than 10% of the protein of the preparations could have been from contaminants. This was true for rats of all ages. Phospholipids from liver or plasma membranes were extracted with 20 vol of $CHCl_3/CH_3OH$, 2/1 (v/v), 0.005% butylated hydroxytoluene for 2 hr at 4° (4). This solvent was used throughout. The suspension was filtered through Whatman No. 43 paper which was previously washed with $CHCl_3/CH_3OH$. The filtrate was maintained at 4°. The filter was extracted with 10 vol of the solvent for 1 hr at room temperature and then with 10 vol of boiling solvent. The extracts were combined and washed with 0.2 vol of 0.05% $CaCl_2$ (5). The aqueous CH_3OH layer was discarded. The volume of $CHCl_3$ was reduced to a yellow oil by rotary evaporation at room temperature. The lipid extract was taken up in the $CHCl_3/CH_3OH$, and stored at −20°.

Silica gel plates (Schleicher and Schuell No. 1500) were activated 1 hr at 110° and transferred to a nitrogen chamber (Brinkmann Co.) for spotting. The lipid extract was applied to the plates as duplicate or triplicate streaks, 10 × 5 mm, containing 0.25–0.40 μmoles lipid phosphorus, with empty lanes, 1 cm wide, between streaks. Phospholipid standards, lysophosphatidyl choline (LPC), sphingomyelin (S), phosphatidyl choline (PC), phosphatidyl inositol (PI), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), cardiolipin (C), and phosphatidic acid (PA) (Applied Science Laboratory) were also spotted on each plate. De Saga type chromatography tanks were lined with Whatman

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3MM paper and saturated with 150 ml of the appropriate solvent. Plates were developed first in hexane to separate neutral lipids from phospholipids, dried for 10 min in a vacuum desiccator over Drierite ($CaSO_4$), and rechromatographed in $CHCl_3/CH_3OH/CH_3COOH/H_2O$, 75/45/12/6 (v/v) (6) until the solvent front was within 1.5 cm of the top of the plate. Spots were detected by a 1 min exposure to iodine vapor. Separations of phospholipids were typical of those described by Skipski, Peterson and Barclay (6). Both spots and blanks from below the origin were scraped off the plate after 1 drop of water was placed on each spot to hold the silica gel together and the phospholipids were eluted from the silica gel with the solvents described by Skipski and Barclay (7). The solvents were removed by lyophilization, using a shell of ice frozen around the tubes to prevent bumping. The lipid phosphorus was measured by the Bartlett procedure (8). Recovery of lipid phosphorus of samples of known phosphorus content spotted, chromatographed and eluted was 89–95%.

TABLE I. Relative Distribution of Liver Phospholipids of Normal Rats Weighing (av) 62 g, 120–130 g, and 400–500 g.

Phospholipid	% Total phospholipid		
	62 g (3)[a]	120–130 g (3)	400–500 g (9)
LPC[b]	4.77 ± 0.60	2.55 ± 0.37	3.19 ± 0.68
S	3.49 ± 0.15	3.48 ± 0.06	4.49 ± 0.27
PC	46.40 ± 0.59	47.96 ± 0.16	47.02 ± 1.56
PI	6.42 ± 0.34	8.24 ± 0.43	8.68 ± 0.95
PS	4.62 ± 0.37	3.82 ± 0.23	4.86 ± 0.25
PE	24.90 ± 0.71	23.61 ± 0.45	23.16 ± 1.10
PA + C	10.63 ± 1.04	10.34 ± 1.85	8.49 ± 0.29
Total[c]	101.23	100.00	99.87

[a] Pooled livers from a single group of rats of average weight 62 g, and a single group of average weight 120–130 g, and from 4 groups of rats of average weight 400–500 g were used. The numbers in parentheses refer to the number of phospholipid determinations. Data from several groups of large rats were combined for statistical purposes.

[b] LPC, lysophosphatidyl choline; S, sphingomyelin; PC, phosphatidyl choline; PI, phosphatidyl inositol; PS, phosphatidyl serine; PE, phosphatidyl ethanolamine; PA, phosphatidic acid; C, cardiolipin; PG, phosphatidyl glycerol.

[c] Micromoles of phospholipid phosphorus.

TABLE II. Relative Distribution of Liver Plasma Membrane Phospholipids of Normal Rats Weighing (av) 62 g, 120–130 g, and 400–500 g.

Phospholipid	% Total phospholipid		
	62 g (3)	120–130 g (3)	400–500 g (16)
LPC[a]	3.72 ± 0.42	6.57 ± 0.96	5.70 ± 0.49
S	18.77 ± 0.17	20.83 ± 1.30	24.54 ± 1.19
PC	33.35 ± 0.41	31.05 ± 1.30	37.54 ± 1.00
PI	7.37 ± 0.35	6.98 ± 0.60	6.83 ± 0.68
PS	10.09 ± 0.38	8.41 ± 0.57	9.57 ± 0.31
PE	18.07 ± 0.26	14.68 ± 0.58[b]	12.41 ± 0.36[b]
C + PA + PG	8.61 ± 0.51	11.47 ± 1.85	3.95 ± 0.37[b]
Total	99.98	99.99	100.54

[a] All abbreviations are as in Table I. Plasma membranes were prepared from the pooled livers of a single group of rats weighing 62 and 120–130 g average, and from the pooled livers of 5 groups of rats weighing 400–500 g.

[b] $p < .001$.

The relative distribution of liver phospholipids of normal rats remained stable during aging of rats (Table I). Similar measures of phospholipid distribution of plasma membranes of these livers, however, indicated that phosphatidyl ethanolamine levels fell in older rats, in marked contrast to other phospholipids which remained unchanged (Table II), within the limits of the method of measurement.

The levels of phosphatidyl ethanolamine in liver plasma membranes decreased during aging from 18% of membrane phospholipid in rats of 62 g to 12% of membrane phospholipid in rats of 500 g (Fig. 1). The levels of membrane phosphatidyl inositol and phosphatidyl choline were unchanged and no differences were apparent in the relative distribution of total liver phospholipid classes.

The phospholipid changes occurring concomitantly with aging in cultured diploid cells as seen elsewhere (1) were not apparent in livers of rats of various ages as reported in the present experiments. However, membrane changes do occur: a decrease of 30% in plasma membrane phosphatidyl ethanolamine with age may have profound effects on various properties of the cell membrane. Gallai-Hatchard and Gray (9) showed that leakage of intracellular enzymes from cells treated

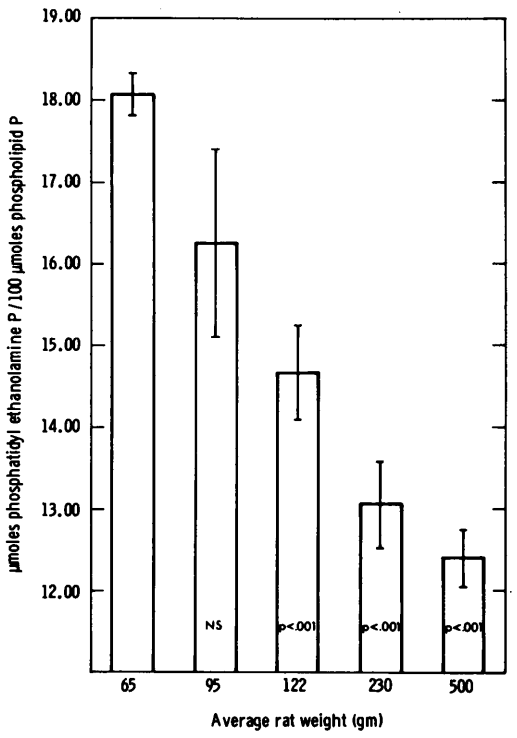

FIG. 1. Changes in phosphatidyl ethanolamine levels of liver plasma membrane from normal rats as a function of rat age. Micromoles of phosphatidyl ethanolamine per 100 μmoles phospholipid phosphorus were determined as described in text. Three determinations were made for each of the single groups of 65, 95 and 122 g (av) rats, 7 for 3 groups of 230 g rats and 16 for 5 groups of 400–500 g rats. Plasma membranes were prepared from the pooled livers of two or more rats. NS = not significant.

with phospholipase A_2 was correlated with the rate of hydrolysis of phosphatidyl ethanolamine but not other phospholipids. In addition phosphatidyl ethanolamine may contribute to the conformation of transport or receptor proteins by maintaining a particular ionic environment near certain segments of the protein by selective binding of specific ions (10, 11).

Treatment of plasma membrane fragments with phospholipase A_2 changed membrane hormone receptors for insulin (12) and glucagon (13). Changes in the affinity of receptor sites for hormones, or the ability of hormone–receptor complexes to transmit signals for metabolic processes may parallel age-dependent changes in membrane phospholipid distribution.

Summary. Phospholipid distribution in livers and liver plasma membrane preparations were determined on rats of various weights. Phosphatidyl ethanolamine levels decreased from 18% of liver membrane phospholipids in rats of 62 g to 12% of membrane phospholipid in rats of 500 g. No differences were apparent in other phospholipid classes.

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